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PROGRESS REPORT

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

A SUMMARY OF PROGRESS
1 January 1965 through 31 December 1965

R. G. Lindberg
J. J. Gambino
PRINCIPAL INVESTIGATORS

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON 25, D. C. 20546

NORTHROP SPACE LABORATORIES
3401 W. BROADWAY
HAWTHORNE, CALIFORNIA 90250

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(Contract NASw-812)

A Summary of Progress

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R. G. Lindberg
J. J. Gambino
Principal Investigators

G. H. Sullivan, M.D.
Director, Life Sciences

Northrop Space Laboratories
3401 W. Broadway
Hawthorne, California 90250

PEROGNATHUS AS AN EXPERIMENTAL TOOL IN SPACE RESEARCH

SUMMARY

The work of this contract was undertaken to establish the feasibility of using pocket mice in biological experiments in space. In the several years that we have been studying this genus, the emphasis may have shifted slightly from time to time, but the primary objective was never forgotten. In this time we have obtained sufficient baseline physiological data to design a self-contained life support and biological data telemetry system capable of maintaining six pocket mice and gathering meaningful physiological data for long periods in space.

In addition, we have sufficient baseline data on blood, chromosomes, circadian rhythms, hibernation and radiation response in at least two species of the genus, P. longimembris and P. formosus, to allow their use in a variety of experiments. We feel that pocket mice can now be used with confidence in many experiments in which conventional mice, rats, or hamsters may be used to study the effect of space conditions on mammals. The pocket mouse still has the added advantages of small body size and low life support requirements that were promulgated early in these investigations.

Brown fat deposits in Perognathus indicate that it is similar to other hibernating mammals in this respect. More work needs to be done on the hibernation characteristics of this genus; nevertheless, present indications are that it can be used advantageously in space probes that may necessitate a hibernating mammal as the experimental subject.

Most promising, perhaps, has been the successful laboratory breeding of pocket mice. It is anticipated that a breeding colony can now be established providing large numbers of genetically stable strains

of pocket mice for all kinds of biological research. Hopefully, the increased availability of this very remarkable rodent will widen their use in laboratories throughout the world, and thereby increase the rate at which knowledge of pocket mouse physiology is accumulated.

The following pages summarize accomplishments under Contract NASw-812, January-December 1965. Sections I and II are papers on breeding and growth, which have been prepared for publication. Section III summarizes both published and unpublished work on pocket mouse radiation biology.

BREEDING

Laboratory Breeding of the Little Pocket Mouse, *Perognathus longimembris*

P. Hayden, J. J. Gambino, and R. G. Lindberg

LABORATORY BREEDING OF THE LITTLE POCKET MOUSE, *PEROGNATHUS LONGIMEMBRIS**

P. Hayden, J. J. Gambino, and R. G. Lindberg

ABSTRACT

A group of 160 females and 40 male captive pocket mice (*Perognathus longimembris*) were examined routinely for reproductive activity during January to June, 1965. Limited observations were made throughout the summer and fall. Fifty-eight percent of the females showed one or more estrous cycles during the routine inspection. Most exhibited polyestrous cycles of approximately 10 days. The incidence of first estrus was highest in March. Breeding was accomplished by pairing animals judged by external signs to be receptive. A total of 217 matings were attempted and 57 litters were produced. Five mice produced two litters each. Litters occurred from late March through September. Males made sexually active with chorionic gonadotropin sired several litters. Information on hormone treatment, estrus, estrous cycles, post-partum and juvenile estrus, mating, gestation, maternal care and nest building is presented.

*Prepared for submission to *Journal of Mammalogy*

INTRODUCTION

The only species of Perognathus cited in the literature as having been bred in captivity are P. californicus and P. flavus (Eisenberg and Isaac, 1963). Because of the aggressiveness of Perognathus, these matings were accomplished only by careful pairing methods and control of environmental conditions. Prediction of female receptivity, size of the mating pens and duration of pairing were extremely important.

There has been marginal success in breeding other captive heteromyid rodents, Dipodomys spp. and Liomys pictus (Butterworth, 1961; Chew, 1958; Day et al., 1956; Eisenberg and Isaac, 1963). Dipodomys appear somewhat easier to breed than Perognathus, although both genera have solitary habits and strong intraspecific aggression. These behavior patterns present the major obstacles to laboratory matings, and successful breeding depends upon modification of the usual techniques employed for breeding laboratory animals (Day et al., 1956).

Efforts to breed Perognathus in this laboratory have emphasized particularly routine observations of natural estrous cycles in a large number of captive animals as a basis for determining most favorable mating time, and induction of sexual activity in males by hormone treatment.

MATERIALS AND METHODS

Holding Facilities and Animal Husbandry: A group of 160 female and 40 male pocket mice were trapped during the spring of 1964 in the vicinity of Whitewater Canyon, approximately ten miles east of Palm Springs, California. The mice were kept individually in gallon jars which contained two inches of sand.

A diet of equal parts hulled sunflower seeds, parakeet seed mix, rye, wheat and rye grass seed maintained the mice in good health, as judged by sleek coats, bright eyes and normal activity. However, there was a question whether this diet lacked a factor necessary for breeding. In the field such a factor may be present in the succulent plant materials that are present during the natural breeding season. Therefore, rye grass seed soaked in water soluble vitamins (Avitron, pet vitamins) was added to the diet. In addition, freshly cut vegetation, consisting of about equal parts unidentified grasses and weeds were given every other morning. The grass seed and fresh vegetation were avidly consumed.

The temperature was maintained between 20-23°C with relative humidity between 50-70%. Overhead fluorescent lights were on from 0600 to 1800 hours. During the dark portion of the light regime, a 7-watt light was on to provide minimum night-time illumination.

Observation Routine: The 160 females were separated into two equal groups for convenience of handling and data acquisition. One group was periodically weighed during the first portion of the study; otherwise, the groups were handled and treated similarly.

Routine observation of genitalia was begun in January, 1965. At first, all females were examined every third day; beginning in February, sexually active females were examined every day. The entire group was examined every 3-7 days, and sexually active mice were added to the daily inspection group.

Beginning in late June, the inspection routine was limited to only those females that had produced litters. Because of somewhat intermittent observations, the data for this period is not included in the graphic presentation of estrous cycles.

Breeding Chambers: Three kinds of breeding chambers were used: 15-gallon glass aquaria; rectangular boxes (8' x 1.5' x 1.5'); and square, compartmented boxes (4' x 4' x 1.5'). The latter cages had six peripheral compartments, opening into a central area. This allowed animals to establish home territories in the six outer chambers and use the central area for breeding. The long boxes had no dividers. All chambers had 3-4 inches of desert sand and rocks. The boxes were provided with lengths of metal and plastic tubing of various diameters to give many places for the animals to hide.

Pairing Methods: The method of selecting animals for pairing was very direct. Females who were judged to be receptive by external signs were placed with males with large palpable testes. The pairs were observed for 15-20 minutes to determine whether they were aggressive. Sometimes animals could be switched around to match "personalities", but this was seldom successful. If the male was pugnacious or reacted violently to the female, he was removed immediately. If the paired mice seemed compatible, they were left together 3-4 hours, overnight, or sometimes longer. The female was then placed in an isolation cage for periodic observation.

Hormone Injection: The hormone chorionic gonadotropin was used to stimulate both males and females. The lyophilized CGH (Calbiochem, 3625 Medford St., L.A., 90063, Calif.) was dissolved and diluted with physiological saline to a concentration of 2000 units/ml and administered subdermally with a micrometer syringe. Doses of 100 units per day were given to a sexually inactive group of 10 females and 10 males in June, 1965. This group was observed for changes in external genitalia. A group of eight females were injected and ovaries taken for histological examination.

Several other groups of females were treated with CGH and paired with untreated sexually active males.

RESULTS

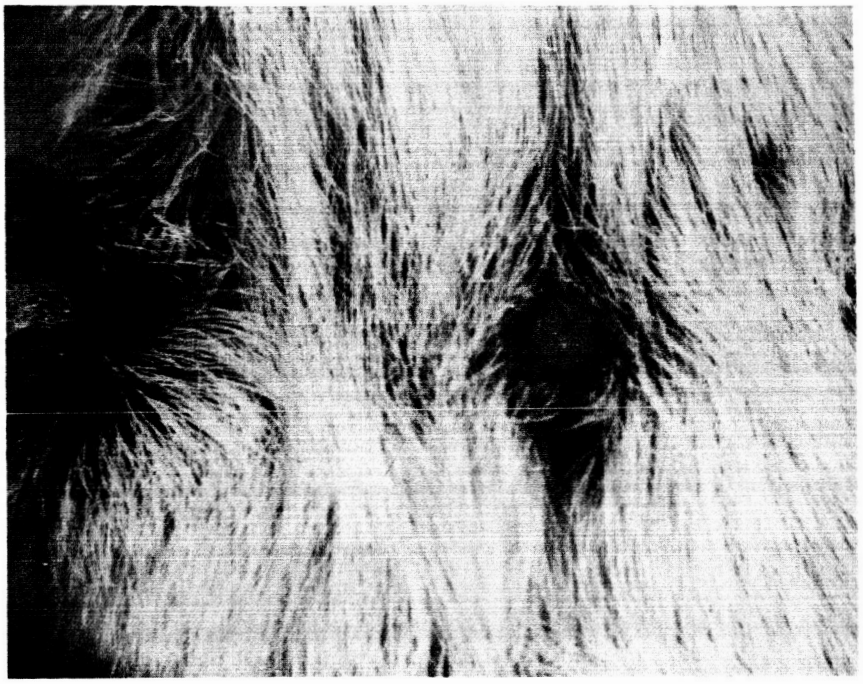
Estrous Cycle: In our captive P. longimembris, estrous cycles started in mid-January and have continued in some animals through December (time of this writing). An estrous cycle is completed in about ten days.

The times of the first observed estrous periods, total number of estrous cycles, and number of litters are presented in Table 1. The incidence of first estrous periods increased from late January to a maximum in March and then gradually declined to June. The sexually active females were observed to have an average of 3.8 estruses, with a range of 1 to 11. At least one full estrous cycle was observed in 58% of the group, with 42% remaining inactive during the five-month daily observation period.

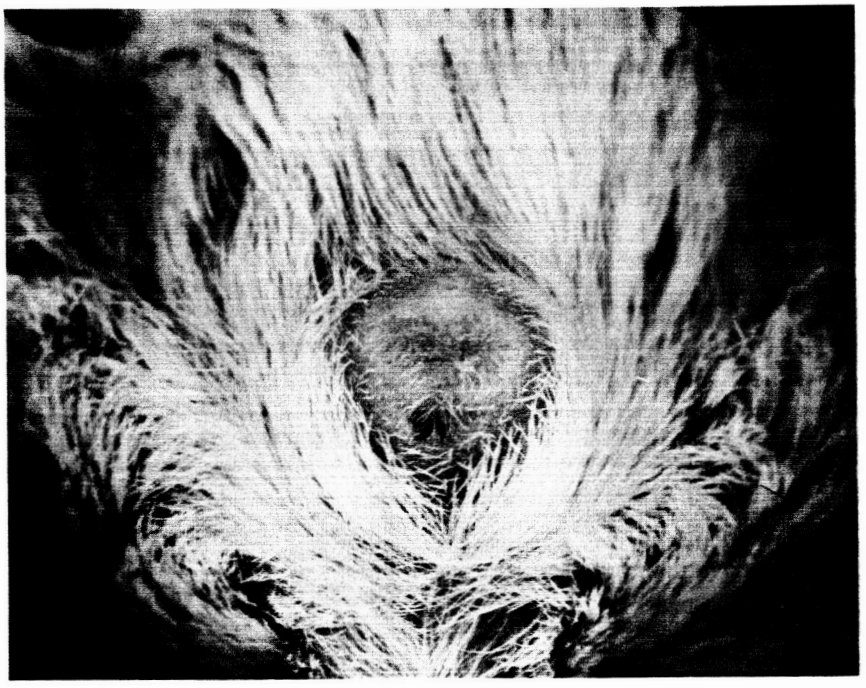
During the anestrus portion of the year, the vaginal orifice is completely regressed and sealed with epithelium (Fig. 1-A). During the polyestrus portion, the orifice is alternately open and closed, with vaginal walls oppressed or sealed with epithelium. The vulva swells during proestrus, 1-2 days before estrus (Fig. 1-B). The vaginal orifice remains sealed during proestrus, but a characteristic transverse line is evident in the vulva. During estrus, the vaginal orifice is open, with the edges much enlarged and evaginated to various degrees (Fig. 2-A). This condition lasts from a few hours to a day. During metestrus, the external genitalia regress and the vaginal lining sloughs off (Fig. 2-B). The sloughed material and mucous form a plug which is retained in the vagina from one to five days. One of these plugs was removed intact from an animal. The consolidated material was a cast of the vaginal cavity

TABLE 1. Summary of Observed Estrous Periods and Litters of 160 Perognathus longimembris.

Time Period	No. First Estrus		Total No. Estrous Periods	No. Litters
Jan 1 - 15	0	6	0	0
16 - 31	6		6	0
Feb 1 - 14	5	18	13	0
15 - 28	13		21	0
Mar 1 - 15	14	32	37	0
16 - 31	18		42	2
Apr 1 - 15	5	23	43	0
16 - 30	18		53	2
May 1 - 15	8	11	62	8
16 - 31	3		24	9
June 1 - 15	3	3	33	14
16 - 25	0		21	13
June 26 - July 26	No obs		No obs	9
TOTAL	93		355	57

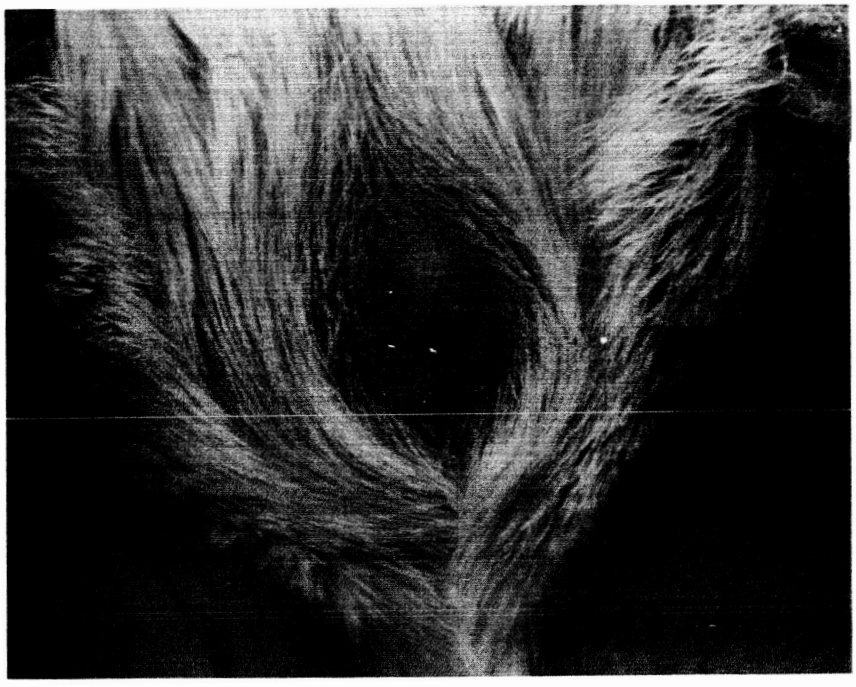


A. Anestrus - not swollen, edges of vagina closely oppressed or sealed

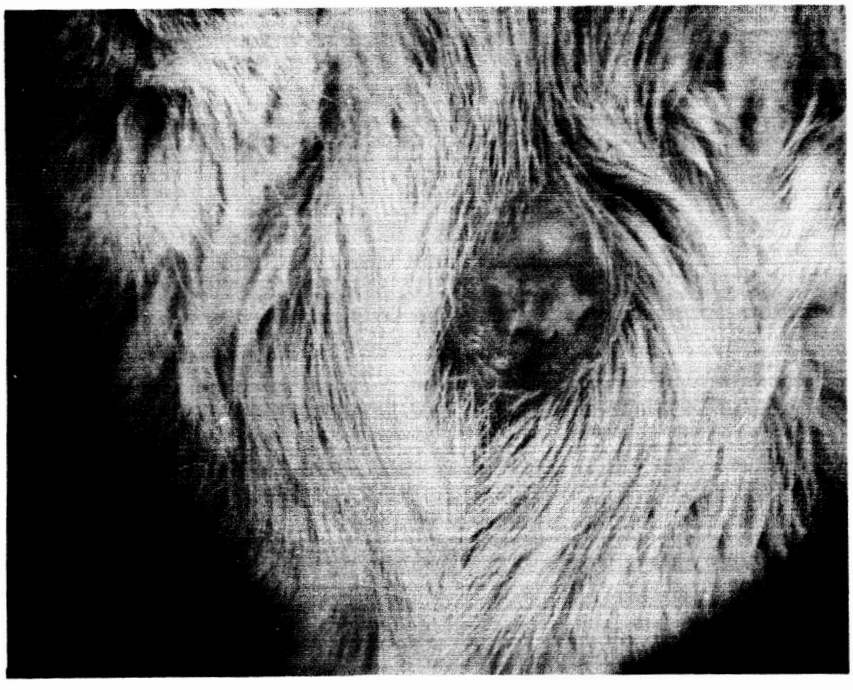


B. Proestrus - swollen but still sealed, future vaginal orifice well defined

Figure 1. Genitalia of female Perognathus longimembris



A. Estrus - swollen, vaginal orifice open, edges evaginated (not well shown in picture)



B. Metestrus - sloughed lining and mucous plug consolidated, swelling diminished

Figure 2. Genitalia of female Perognathus longimembris

complete with a bifurcated tip, which indicated that sloughing of the lining involved at least a portion of the uterine horns.

A post-partum estrus was documented in this species. In two instances females ate their offspring shortly after birth and were returned immediately to routine observation. Estrus was observed at two days post-parturition in one of these animals and at four days in the other. Both females were paired again, and the former bore a litter.

One female born in the laboratory was noted to have swollen external genitalia, but a sealed vaginal opening at 41 days after birth. The next day a well-formed vaginal plug was observed, indicating that an estrous cycle had been completed. This suggests that sexual maturity may be reached in P. longimembris females as early as 41 days. This individual was again in estrus 30 days later and was paired with a male, but no litter was produced.

A gravid juvenile P. formosus was field-captured and littered in the laboratory but failed to take care of the neonates. This animal was judged to be juvenile by the grey color of the coat and was evidently born early in the spring.

These data indicate that the early young of the year are capable of breeding the same year they were produced.

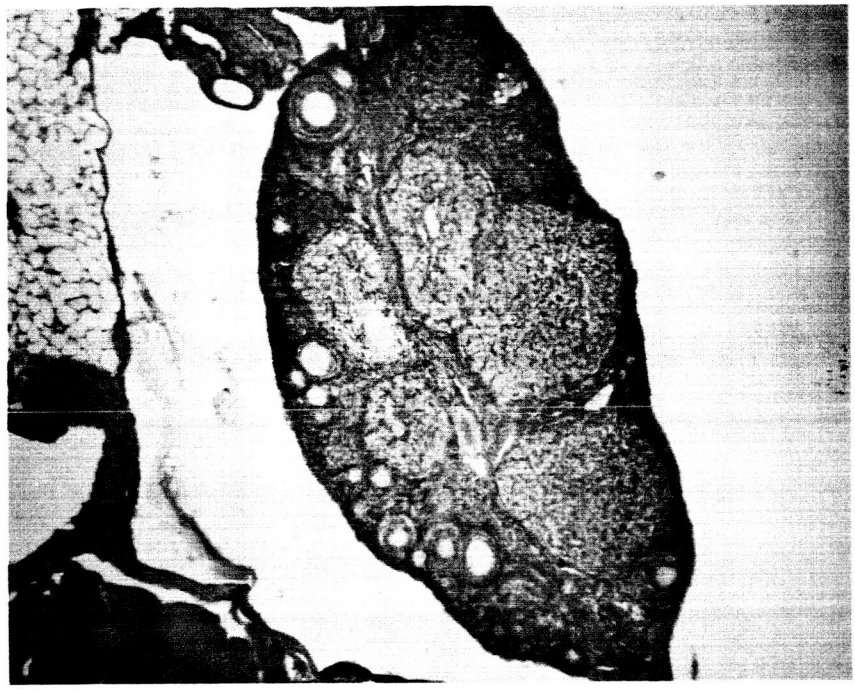
Reproductively Active Males: Although males with conspicuous descended testes have been observed in the field, none were observed during this laboratory investigation. Some of the captive animals and the hormone-treated males had enlarged testes that could be forced down into the scrotum and be retained for several seconds. The abdominal position of the testes does not prevent production of viable sperm, as evidenced by successful matings.

Along with testicular enlargement there is an increase in the amount of vascular tissue surrounding the os baculum, giving a rugose texture to the penis. In the inactive male, the penis is characteristically thin and pale with little vascularization.

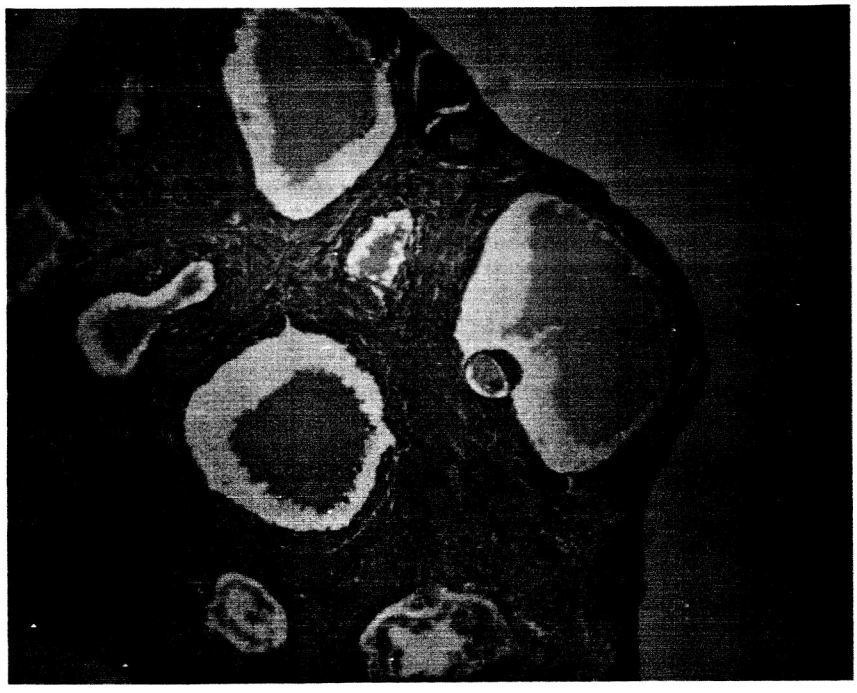
Induction of Sexual Activity: In an early attempt at induction of sexual activity, estradiol and progesterone were used without success. However, CGH was found to give an externally visible effect in females after about a week and in males after 10-12 days.

Of the ten anestrus females that were injected with CGH, six had open vaginal orifices before seven days and remained so for 2-5 days; two opened after seven days, and two were still sealed after 16 days. In these hormone-induced openings of the vagina, swelling and evagination of the vulva did not occur or was much less than in normal estruses. Several other groups of females were given 100 units/day for seven days and paired with both normal and hormone-induced males, but none of the females were receptive.

After seven injections of 100 units CGH/dose, several females who exhibited opening of the vagina were sacrificed for autopsy and ovarian tissue. Sections of ovaries are shown in Figure 3. In the uninjected



A. Normal - untreated animal in anestrus



B. Active - hormone-treated animal shows externally visible signs of estrus when sacrificed

Figure 3. Ovaries of Perognathus longimembris - low power view (120X) Note developing Graafian follicle in hormone-treated animal.

control animals, the ovaries were small and had no obvious developing ova; the uteri were transparent and flaccid. The ovaries of the injected animals were much enlarged with many developing ova, and uteri were well-developed.

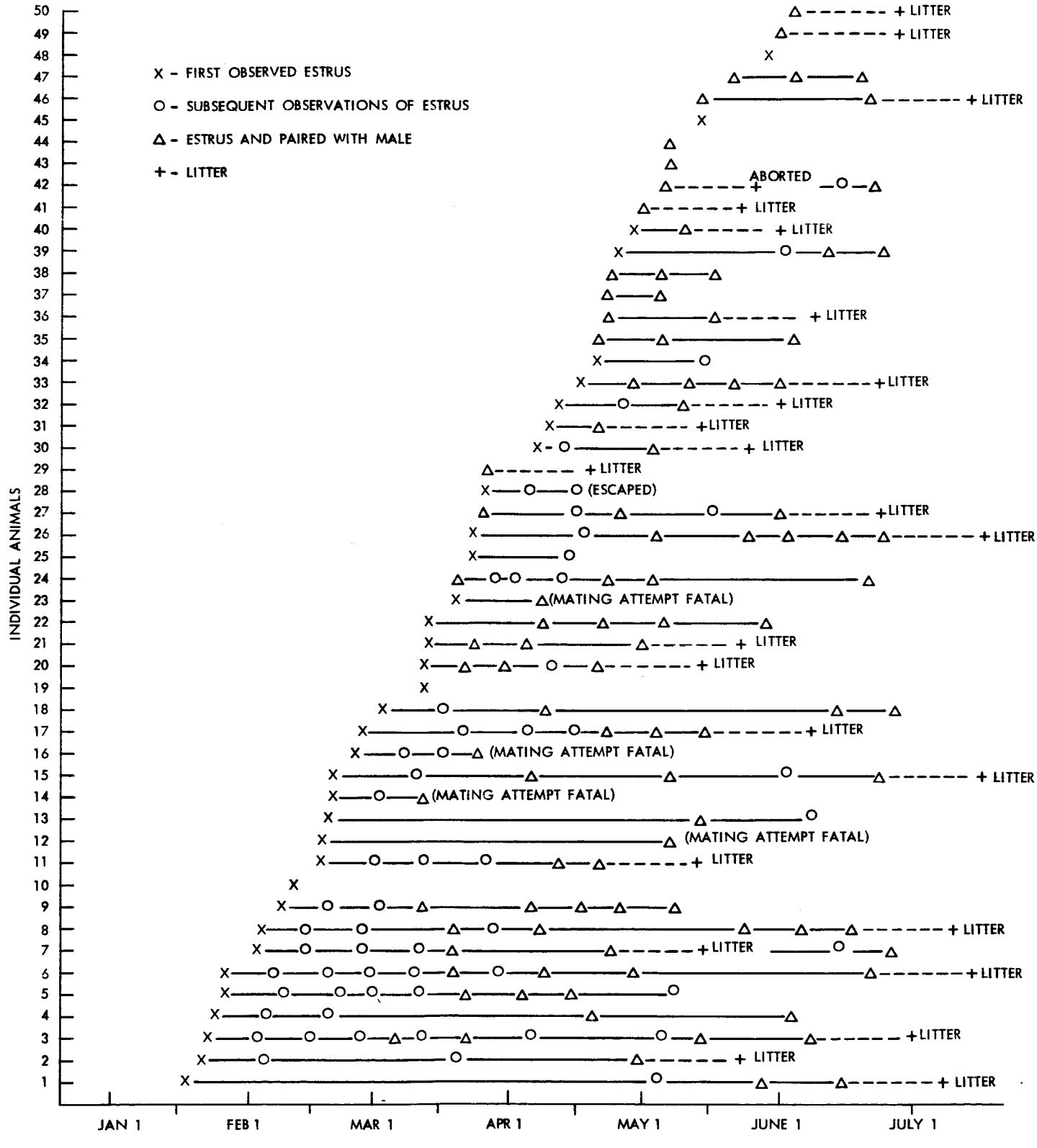
The testes of all injected males became enlarged and could be palpated after about 12 days, but the testes did not become scrotal. Viable sperm developed in hormone-induced males, since they have sired several litters with normal females.

Breeding: Observations on frequencies of estrus, mating attempts and litter production are summarized in Figures 4 and 5. These represent two randomly chosen groups of 80 females each; mice in Figure 4 were weighed biweekly during the first six weeks, while those of Figure 5 were not weighed. Estrus began earlier in the weighed group (January), than in the unweighed group (February). More of the weighed mice showed estrus (50 of 80) than of the unweighed (43 of 80). Pairings of females in estrus with males were not attempted until 1 March.

Daily inspections of reproductively active females detected 355 estrus cycles in progress; 217 matings were made, which is a utilization of 61% of the potential receptive periods. A total of 57 litters resulted from the 217 pairings, which is a success of 26%. This is considered a high level of achievement, since sexual receptivity occurs only during the first half of estrus.

Litter size averaged four and ranged from one to six. The sex ratio of those young weaned by 30 June was 30 ♀♀ to 34 ♂♂ (47:53). In eleven instances, the first observed estrus and pairing resulted in the production of a litter. Five females have produced two litters each. The 52 females that bore litters were kept under observation biweekly after June 25.

Figure 4. Estrus periodicities, breeding attempts and litter production in reproductively active females from a group of 80 P. longimembris. Animals weighed routinely.


X - FIRST OBSERVED ESTRUS
O - SUBSEQUENT OBSERVATIONS OF ESTRUS
Δ - ESTRUS AND PAIRED WITH MALE
+ - LITTER
INDIVIDUAL ANIMALS
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
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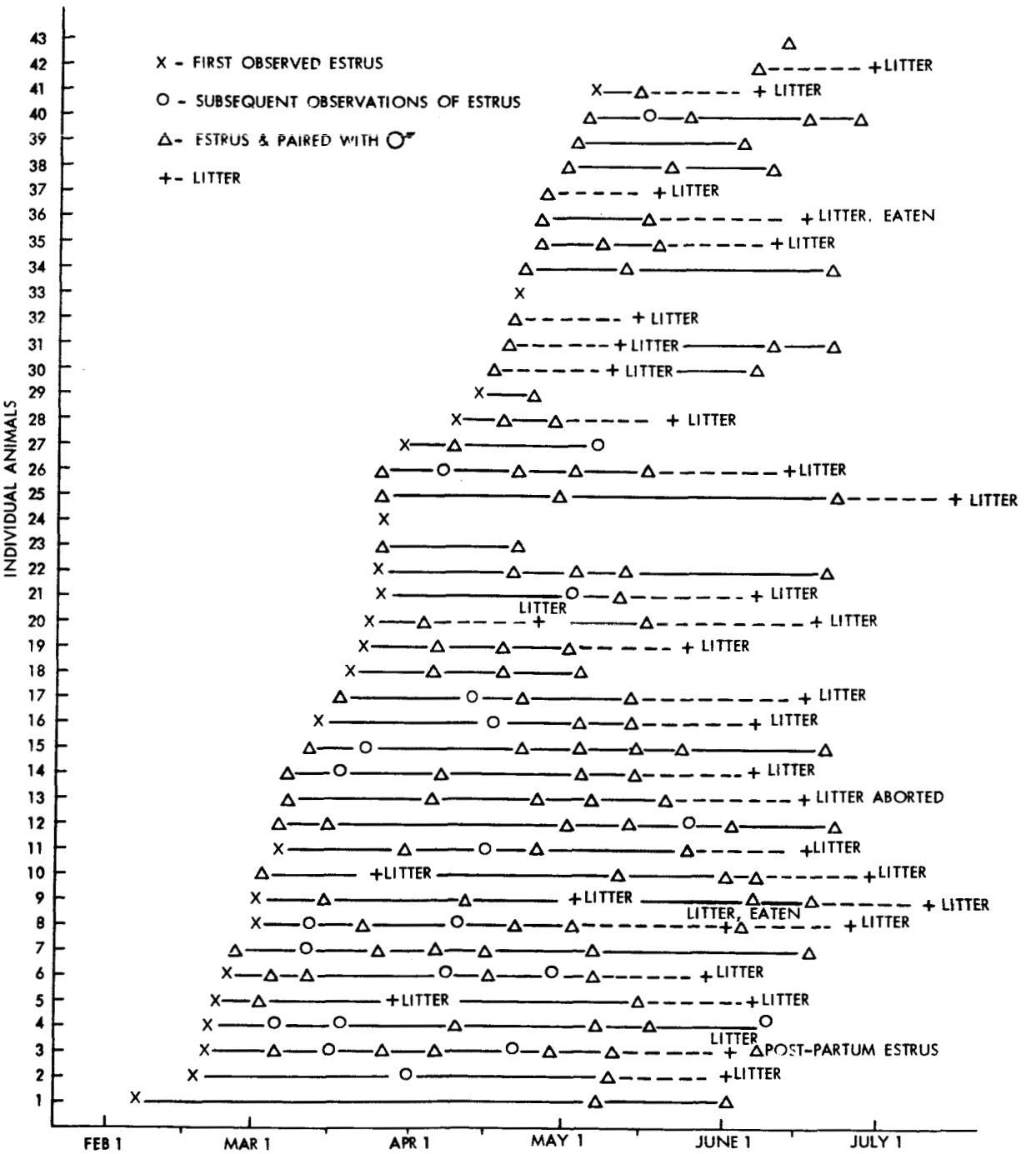
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
JAN 1
FEB 1
MAR 1
APR 1
MAY 1
JUNE 1
JULY 1
+ LITTER
ABORTED
(ESCAPED)
(MATING ATTEMPT FATAL)

Figure 5. Estrus periodicities, breeding attempts and litter production in reproductively active females from a group of 80 P. longimembris. Animals not weighed.


X - FIRST OBSERVED ESTRUS
O - SUBSEQUENT OBSERVATIONS OF ESTRUS
Δ- ESTRUS & PAIRED WITH ♂
+- LITTER
INDIVIDUAL ANIMALS
FEB 1
MAR 1
APR 1
MAY 1
JUNE 1
JULY 1
+LITTER
+ LITTER
+ LITTER, EATEN
+ LITTER ABORTED
LITTER, EATEN
POST-PARTUM ESTRUS

They continued to undergo estrus periods to the time of this writing, Dec. 1, 1965. Three more litters were produced, the last on 29 Sept.

A total of 26 males sired the 57 litters; however, five males sired 52% of the litters (30 of 57). After several matings these males had short and severely scarred tails, indicative of the aggressive behavior of the females even at a "compatible time".

Fatalities occurred in eight matings (4 ♀♀ and 4 ♂♂). In three cases the animals were partially eaten. Dominated animals were in a state of torpor, although they seemed only superficially wounded by bites on back and tail.

Copulation: The copulatory behavior of P. longimembris is sufficiently different from other species of Perognathus to warrant description (Eisenberg, 1963). In one typical observed mating, the two animals approached one another directly after being placed in the breeding cage. After one nose-to-nose contact and a simultaneous leap, copulation ensued. The male mounted from the rear and both animals fell on their sides. Copulation was accomplished in this position. The male did not bite or grasp with his front feet, but grasped the female's tail with one of his hind feet and thrust rapidly. Mounts lasted no longer than 4-5 seconds and appeared to be terminated by the female. In a ten-minute period, there were 20-25 mounts with an undetermined number of intromissions. During the final encounter, the female bit the male on the head, which precipitated a brief kicking and scratching battle. The male ran to a corner, laid down, and remained motionless. The female dug in the sand and preened herself. Twice the female approached the male and attempted to mount him, but he did not move and responded only by squeaking.

The female was examined immediately after copulation. Her external genitalia were no longer swollen and evaginated as before copulation, and the vaginal orifice was sealed with a crust. Egoscue (personal communication, 1962) noted this immediate disappearance of the vulval swelling after breeding Dipodomys spp. This may indicate that superfecundation is not possible in the heteromyid rodents.

Several other females have been observed to assume a copulatory position (lying on one side with pelvis rotated 90° and one hind leg extended nearly vertical to substrate) when a male approached. This behavior has also been noted when a male was no longer close to the female, but after initial contact had been made.

Gestation and Maternal Care: Gestation periods were definitely established in 31 of the pregnancies. Gestation lasted 22 to 23 days in 74% of these cases; extremes of 21 days and 30 days were recorded. The one animal that littered at 31 days ate the litter shortly after parturition.

Abortions and subsequent eating of the young occurred in five of the 57 litters. Several isolated deaths of newborn mice were observed, but they could not be attributed to maternal neglect.

Maternal care often involved much apparent aimless carrying and shifting of neonates. This continued as late as the third week, when offspring were almost as large as the dam. Even at this age, the young did not struggle when picked up by the dam; rather, they facilitated the action by raising their legs. Litters were weaned at about 30 days.

Nest Building: Pocket mice generally build nests when material is available. Pregnant mice in our colony were provided with dry grass for nest building, and casual observations were made on nest building behavior and nest construction.

Size of nest seemed to vary with the amount of grass available. Fine grass was cut to ½-inch lengths and used for the bulk of the nest, while coarse grass was shredded and used as bedding. The bed area was about 1½ to 2 inches in diameter. If sufficient grass was available, the nest was covered.

Field Samples of P. longimembris: Animals that were trapped in the Whitewater Canyon area and returned to the laboratory during the period March 5 - June 28, 1965 are listed in Table 2. The first gravid female was trapped in mid-April and juveniles began to appear about seven weeks later. By mid-June, juveniles constituted 84% of the catch.

The mean weight of female animals when weighed several days after capture was 8.6 gms (range 6.4 - 12.2 gms). The same animals nine months later weighed 9.4 gms (range 7.4 - 11.0 gms), indicating good adjustment to our laboratory conditions.

DISCUSSION

There is little doubt that the seasonal reproductive cycle of Perognathus in nature is correlated with annual environmental cycles, but is subject to perturbations by local climatic conditions. Chew and Butterworth (1959) made observations of Perognathus and Dipodomys in their ecological study of rodents at Joshua Tree National Monument, California. In this study, they noted pregnant mice, males with scrotal testes, and very young animals in February, March and April. French (1964) notes that juvenile pocket mice appear in numbers in June at the Nevada Test Site, suggesting that they are probably born in April or May. Hall (1946) indicates nearly all pregnancies occurred in May. The peak of reproductive activity in these desert rodents has been attributed to seasonal variation in rainfall, plant growth and other ecological factors.

TABLE 2. Field Collected P. longimembris Received in 1965.

Date	No. Received		% Juveniles	% Gravid ♀♀
	♂♂	♀♀		
5 Mar 65	3	4	0	0
10 Mar 65	18	6	0	0
17 Mar 65	3	3	0	0
25 Mar 65	24	16	0	0
19 Apr 65	18	5	0	20%
6 May 65	9	8	0	50%
17 May 65	13	7	0	28%
2 June 65	21	17	18.4%	25%
8 June 65	25	25	52.0%	4%
14 June 65	24	28	84.6%	7%
22 June 65	49	59	60.2%	0
28 June 65	29	29	51.7%	0

It is evident from our observations that initiation of reproductive cycles in laboratory-maintained pocket mice coincides with the natural breeding season, as judged by field collection data. For example, a high incidence of juveniles occurred in June, 1965 field samples. This would suggest that conception occurred in field animals at approximately the same time captive animals in this study had a high incidence of estrous cycles.

In the present study, captive pocket mice previously maintained in a stable laboratory environment for at least eight months produced litters from March through September. This may indicate that preparative processes for breeding are associated with an annual endogenous cycle, but initiation and maintenance of the breeding condition is dependent upon diet.

The earlier onset of estrus in the weighed group in this study (Fig. 1) cannot be explained. Ostensibly, both groups were otherwise treated similarly; however, the difference could have resulted from more subtle differences in housing or handling of the animals, or it may represent random variation.

Laboratory breeding of Perognathus longimembris can be accomplished by proper selection of mating pairs. The key to obtaining sufficient compatible pairs is routine observation of large numbers of both sexes to ascertain their state of reproductive activity. Females in full estrus (i.e., with open vaginal orifice) are placed with males with enlarged testes, resulting in copulation and conception approximately 26 percent of the time.

The use of hormones to induce sexual activity has been marginally successful in females in that a condition resembling estrus has been achieved, but no litters have been produced from treated females. In males, the success of hormone treatment has been evidenced by litters produced.

Obviously, routine observation and selective pairing of large numbers of pocket mice is time consuming and expensive. Establishment of a breeding colony may depend upon whether laboratory-conceived and -reared animals will exhibit modified physiological and behavioral patterns, i.e., continuous estrous cycles and greater compatibility.

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Growth and Development of the Little Pocket Mouse, *Perognathus longimembris*

P. Hayden and J. J. Gambino

ABSTRACT

Growth and development data are presented on 8 litters of Perognathus longimembris. This is the first recorded laboratory breeding of this species. Body weights and measurements of ear, hind foot, tail and total body length were taken on 26 individuals. At birth, body weight is about 1.3 g; the skin is nearly transparent and naked, with eyes and ears sealed. There are no cheek pouches at birth. By day 5 the pinnae are unfolded, but the meatus remains sealed. Sparse hair is evident at day 8; by day 14 a full juvenile coat is attained, and the eyes are open. Cheek pouches start to invaginate at about day 3 and are functional by day 14. About 65% of adult weight (8-10 gms) is attained in 21 days, but the hind foot reaches near adult-size during this period. Semilogarithmic plots of growth measurements showed polyphasic growth with initial instantaneous growth rates which varied from 4.4% for total length to 14.8% for ear.

GROWTH AND DEVELOPMENT OF THE LITTLE POCKET MOUSE, *PEROGNATHUS LONGIMEMBRIS**

P. Hayden and J. J. Gambino

INTRODUCTION

Growth and development data for the Heteromyid rodents are very limited. This group contains kangaroo rats, pocket mice and kangaroo mice, nearly all of which are noted for their adptation to an arid environment. These animals are solitary in nature and exhibit strong intraspecific aggression. Most attempts to breed them in captivity have been unsuccessful. However, several species of the genus *Dipodomys* have been bred in the laboratory, and various details of mating behavior, growth and development are available (Day *et al*, 1956; Chew, 1958; Chew and Butterworth, 1959; Butterworth, 1961a,b).

More recently, several species of pocket mice (genus *Perognathus*) have been mated under laboratory conditions (Eisenberg and Isaac, 1963). However, only limited growth data is presented on 3 litters of *P. californicus*, one litter of *P. penicillatus* and one litter of *P. flavus*. Successful breeding of *P. longimembris* and preliminary data on growth and development were reported earlier from this laboratory (Hayden *et al*, 1965). The current report presents representative data from 61 litters of *P. longimembris* that have been bred in our laboratory between April and September, 1965.

*Prepared for submission to *Growth*

METHODS AND MATERIALS

Observations of growth and development were made on eight litters with a total of 26 individuals. One of the litters was from a pregnant female trapped in the field, while the rest were laboratory-bred. All of the females were from the Sonoran Desert of California (Whitewater Canyon area, about 10 miles east of Palm Springs). The observation group was reduced to 24 animals at 14 days and to 22 at 32 days because of death and escape of individuals.

Females and their litters were housed in galvanized boxes, 8" x 11" x 6.5" with screen wire tops. Sand approximately 1-2 inches deep was provided in the cage for digging and grooming. A container (pint can, milk carton, etc.) was provided for nesting. Dry grass (timothy and rye or bermuda) was placed in the container for nest material. Torn paper towels were used in addition to the grass in several cases. Gravid females were placed in the maternity cages 2-8 days prior to expected parturition date to allow them to acclimate to the new environment. If the female appeared nervous or easily agitated, the screen top was covered with a towel, and her cage placed in a more isolated position in the animal room.

The diet was provided ad libitum and consisted of a mixture of about equal parts: hulled sunflower seed, rye grain, oat groats, hulled millet, parakeet mix, rye grass seed and rye grass seed enriched with water soluble vitamins (Avitron, trade name). Pieces of raw carrots were provided every other day.

Litters were handled only after the hands were washed thoroughly with soap and water, rinsed in ethyl alcohol and dried. The first few litters were handled hesitantly, but it was found that newborn mice could be

manipulated from the first day of life with no apparent adverse effects.

Measurements were taken routinely on specific days of the week. However, since litters were added to the sample group as they were born, animals in the various litters were not all measured at precisely the same age. For this reason, plotted values do not represent the same number of individuals. This fact was taken into consideration by weighting the points when the curves were visually fitted. The following measurements were made on all animals: body weight, total body length, tail length, hind foot length and ear length. Tail and body measurements were taken with a flexible plastic millimeter ruler, while hind foot and ear were taken with vernier calipers. Linear measurements were read to 0.5 mm (except ear, to 0.1 mm), weights to 0.01 gm.

Measurements of total body length were taken on active juveniles while the animal, held by the tail, extended itself in an escape attempt. Tail measurements were taken from the same position, using slight pressure of the ruler against the base of the tail. Such measurements on live animals are subject to inherent errors and do not represent absolute values.

Measurements were analyzed as in Brody (1945). Measurement values were plotted on a logarithmic scale versus age on an arithmetic scale. Linear segments of such a plot indicate periods when growth increments were a constant percentage of previous size. From these linear sections, instantaneous growth rates were calculated as:

$$k = \frac{\ln m_2 - \ln m_1}{t_2 - t_1}$$

The value $\underline{k}$ is the instantaneous percentage rate of growth for the unit of time in which t_2 and t_1 are expressed; $\ln m_2$ and $\ln m_1$ are natural logarithms of the measurements made at t_1 and t_2.

OBSERVATIONS AND DISCUSSION

General Development: At birth, the skin is hairless, pink, wrinkled and nearly transparent. The internal organs can be clearly seen through the ventral surface, as can blood vessels, brain and sutures in the skull. Vibrissae are present on the snout at birth and are about 1-1.5 mm long.

Dark pigmentation starts to appear on the head and back at 4-6 days and eventually covers the body down to the lateral line area of the adult. Sparse dark gray hair appears on the pigmented area of the dorsum at 7-9 days with rather coarse white hair on the flanks and legs. By day 13-15 a full coat of juvenile pelage is present, gray on the back, whitish underside and with a buff cast in the head region. The adult colored pelage of pinkish or ochraceous-buff overlaid with blackish hairs on the dorsal surface and pale tawny to buffy white on the ventral surface starts to appear between 29-40 days. Color changes appear first on the back of the head or under the eyes.

The ears, sealed at birth, appear as protuberances. At 3-4 days, a groove deepens on the anterior surface of the auditory protuberance and forms the pinnae. By day 5, all pinnae are unfolded and are about 1 mm long. The meatus is still closed at 13 days, and the exact time of its opening was not determined.

The eyes are sealed at birth and appear as large, heavily pigmented areas behind the thin integument. Eyelids start to develop at 6-7 days and are well formed by day 14. In most of the litters, the eyes were open by day 14-15, but in one litter they did not open until day 18. These data agree with those presented for P. californicus, penicillatus and flavus (Eisenberg and Isaac, 1963).

The toenails are not evident at birth but are quite distinct by day 3. The incisors penetrate the gums as early as day 5 and are evident in all young by day 11. Cheek pouches are not present in the newborn. By day 3, creases lateral to the mouth are present. These creases gradually invaginate and by day 19 are approximately 1/8" deep. The pouches increase in depth and are lightly haired by the time the juvenile pelage is acquired.

The external genitalia are very similar in both sexes but can be fairly well differentiated by 14 days. One female became sexually active at 60 days (vulva open and swollen). No males had descended testes up to 150 days (April-September).

Behavior: All neonates exhibited the righting reflex and were able to crawl, although very laboriously. Ability to move increased daily and by day 6, one animal was noted to perform digging motions involving coordinated movement of fore and hind legs. This occurred when the animal was placed upon a metal pan in preparation for weighing and was obviously disturbed by the cold metal. By day 8, the young moved about the cage freely and by day 12 were gamboling about, even though their eyes were still sealed.

Young mice were noted eating raw carrot at 10 days, and seeds were found in pouches by day 14; they appear to become self-sufficient at about 18 days. One litter survived when the mother died at 14 days.

Siblings were normally separated and placed in individual containers at about 30 days. On occasion, they were separated as early as 21 days or as late as 42 days. Sibling aggression and resultant injury or death was noted in several litters less than 30 days old.

When neonates were carried by the dam, they drew their legs up close to their body, thereby facilitating her movement around the cage.

This same reflex was noted when the young animals were picked up by the loose skin of the back during measurements.

Weight and Measurement: When weights are plotted on a logarithmic scale, there are four distinct phases of growth during which the percentage of increase per day is constant (see Fig. 2). The first phase of almost 7.4% size increase per day brings the individual to 65% of adult weight in 21 days. P. californicus attains only 39% of adult weight during the same time (Eisenberg and Isaac, 1963). Unfortunately, comparative data for P. flavus, which is similar in size to P. longimembris, are not available, although this species has been bred in captivity (one litter, Eisenberg and Isaac, 1963). The initial growth rates of Dipodomys spp. (Chew and Butterworth, 1959; Butterworth, 1961) are greater, but are not sustained as long as in P. longimembris (11 days at 13.0% vs. 21 days at 7.3%). Kangaroo rats attain about 30-50% of maximum weight during this first 20-day period.

Growth rates for P. longimembris level off by about 50-60 days, at which time near maximum weight has been attained. After that time, body weight increases at a rate of about 0.02% per day. Maximum body weight probably is not a meaningful value, because body weight fluctuates seasonally as well as daily.

Inflections in weight increase are suggested to be associated with milestones in the development of wild rodents. Chew and Butterworth (1963) noted that the first inflection of the curve for the kangaroo rat, D. merriami, coincided with opening of the eyes and ears. No change was observed in P. longimembris at 14-15 days, the time of eyes opening. The first change of growth constants (7.4%-2.3%) did not occur until 21 days, which approximates the time of spontaneous weaning or self-sufficiency.

Increases of other dimensions commonly used as indices of growth are given in Figs. 3-4. Like total body weight, total length, tail, ear and hind foot all had a four-part growth. Total length and tail length show their first growth inflections to lower values at about the 15th day (eye-opening) and the second inflection at the 26-27th. These data agree with those presented for the kangaroo rat, D. merriami (Chew and Butterworth, 1963).

The hind foot attained a near adult-size at about 20 days. This means that when the animal attained about 70% of its total length and about 60% of its weight, it had an adult-size foot. Inflection of growth rate occurred at 10 and 20 days. Although this species is not as dependent upon saltatorial locomotion as the genus Dipodomys, this mode of travel is used during escape attempts and at other times when maximum speed is valuable. The adaptive value of a fast-developing foot is self-evident.

The ear had the highest rate of growth, with a value of about 15% during the first 9 days. The inflections of growth rate are similar to those documented in the hind foot. Nearly 86% of adult size was reached by 25 days, with adult size being attained at about 55 days.

SUMMARY AND CONCLUSIONS

Data are presented on the growth and development of 26 individuals from 8 litters, 22 of which were the result of the first recorded matings of Perognathus longimembris in captivity. Newborn of this species of pocket mouse are naked and have a nearly transparent integument; pigmentation gradually fills the area above the future lateral line and is complete with sparse hair by day 9. Eyes and ears are sealed at birth with the pinnae developing at about day 4. The external meatus opens after day 13.

Eyes usually open between day 14-15. Cheek pouches are not present at birth, but start to develop on day 3 and are functional by day 14.

Semilogarithmic plots of body weight, total length, and lengths of tail, hind foot and ear show polyphasic growth. All show a four-part pattern with initial instantaneous percentage growth rates which vary from 4.4% for total length ot 14.8% for ear. Hind foot reaches near adult size by 20 days.

ACKNOWLEDGMENTS

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Fig. 1 - Development of the Little Pocket Mouse (Perognathus longimembris) from neonate to adult. Age in days is indicated in lower right corner in each sequence. The background grid is 1 cm^2. Note unfolding of auditory pinna between day 1 and 4, and rapid total development of animal between 4 and 9 days.

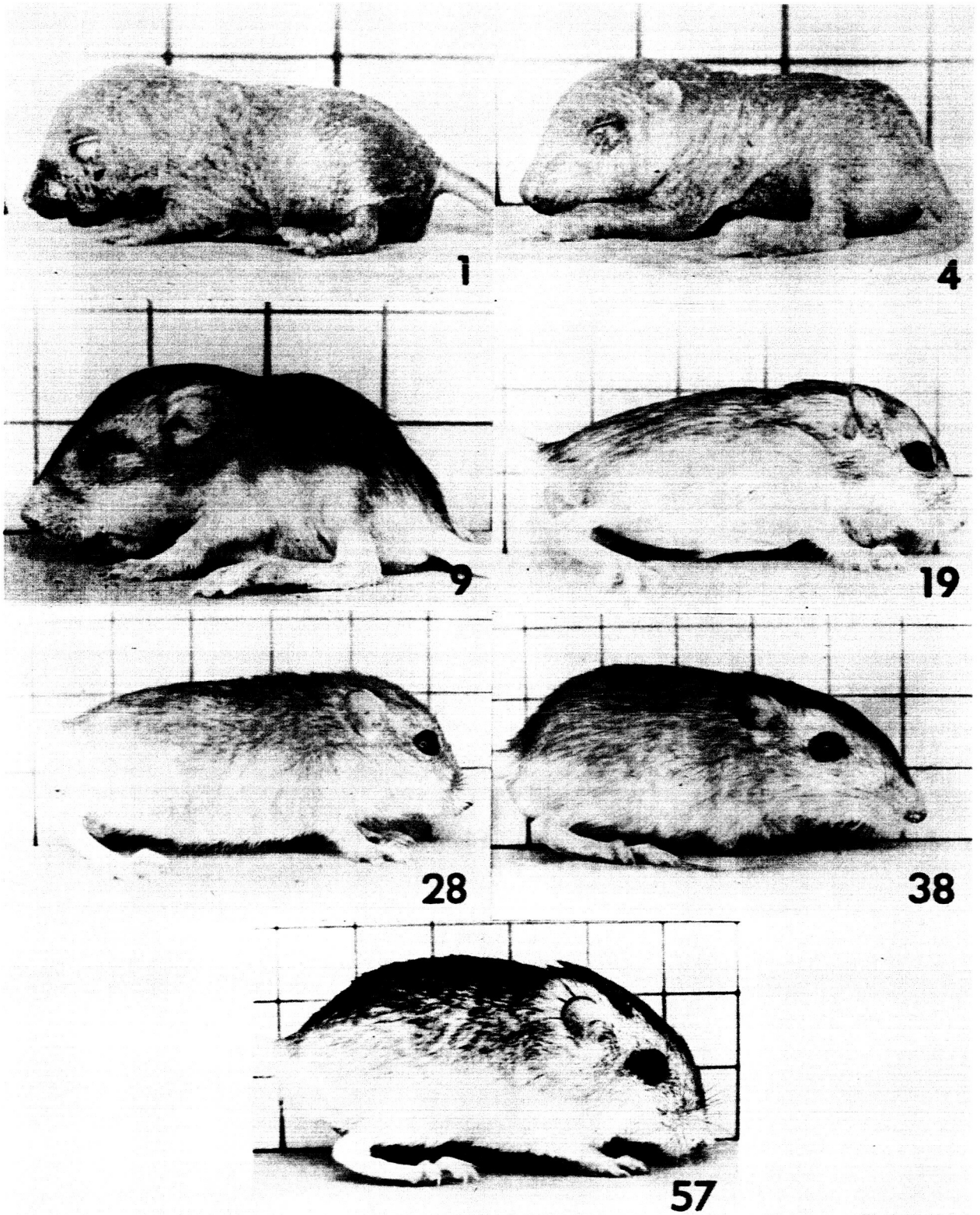

1
4
9
19
28
38
57

Fig. 2 - Semilogarithmic plot of weight increase with time of the Pocket Mouse (P. longimembris). Values above linear segments of plot are instantaneous growth rates.

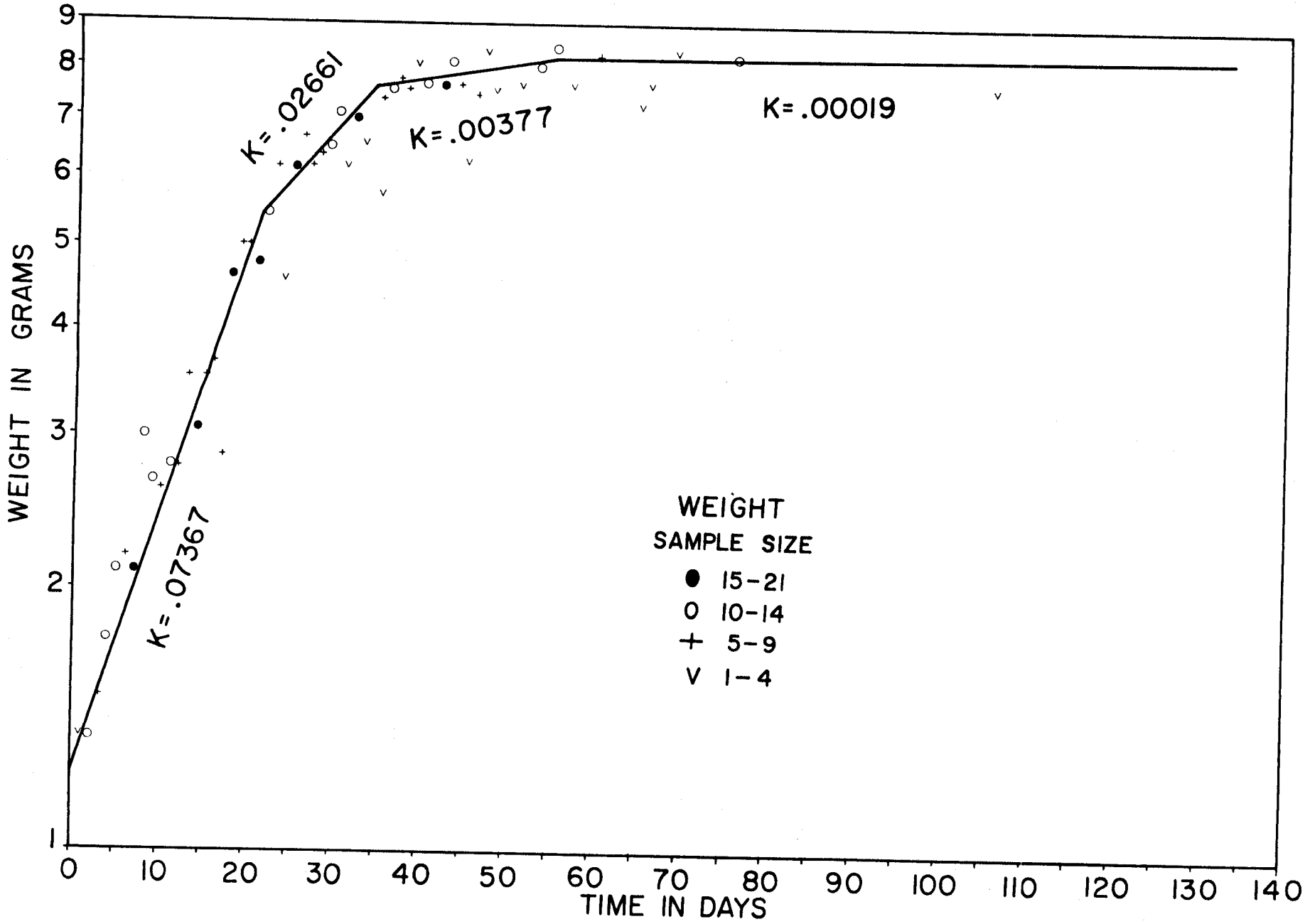
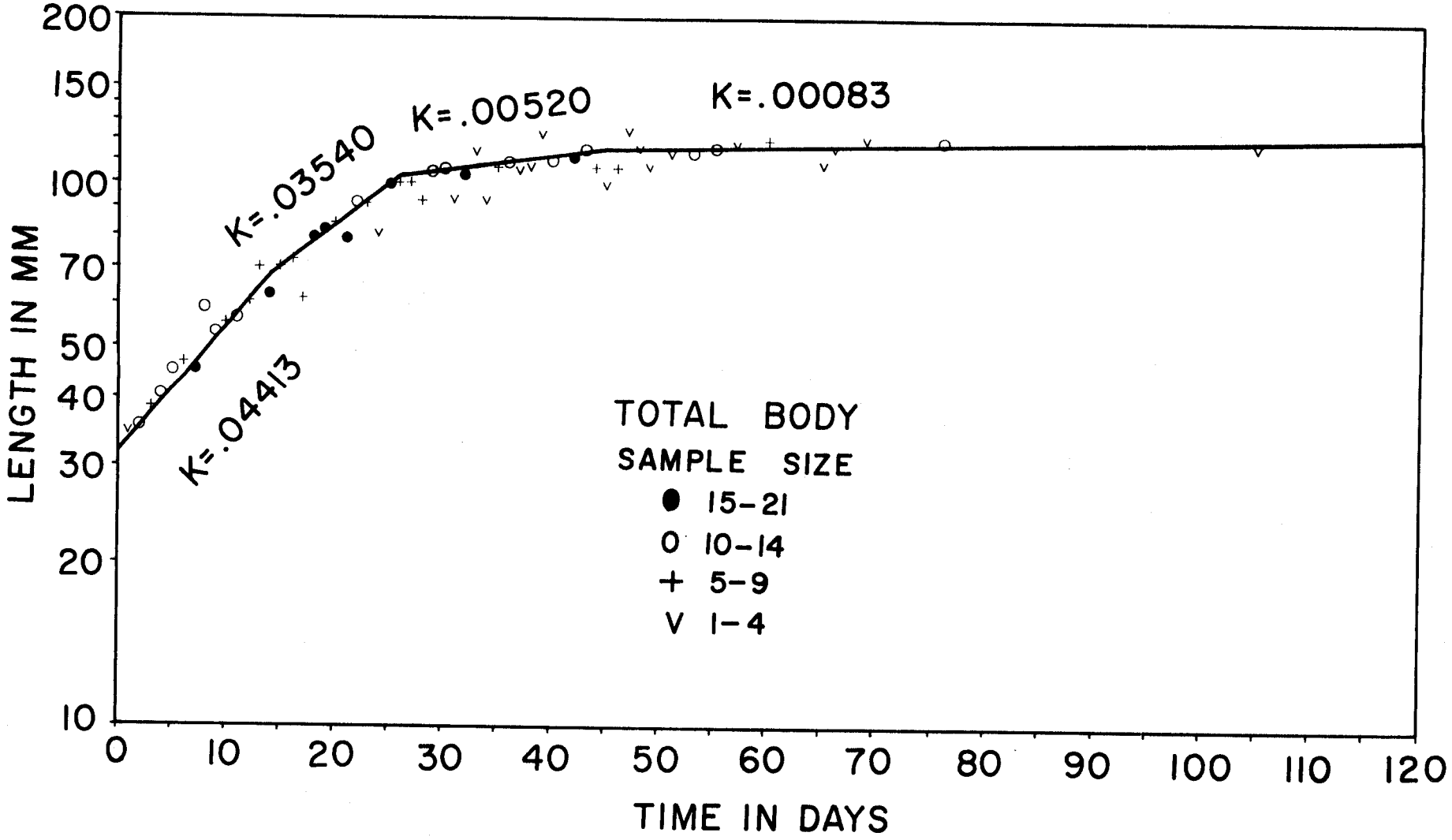
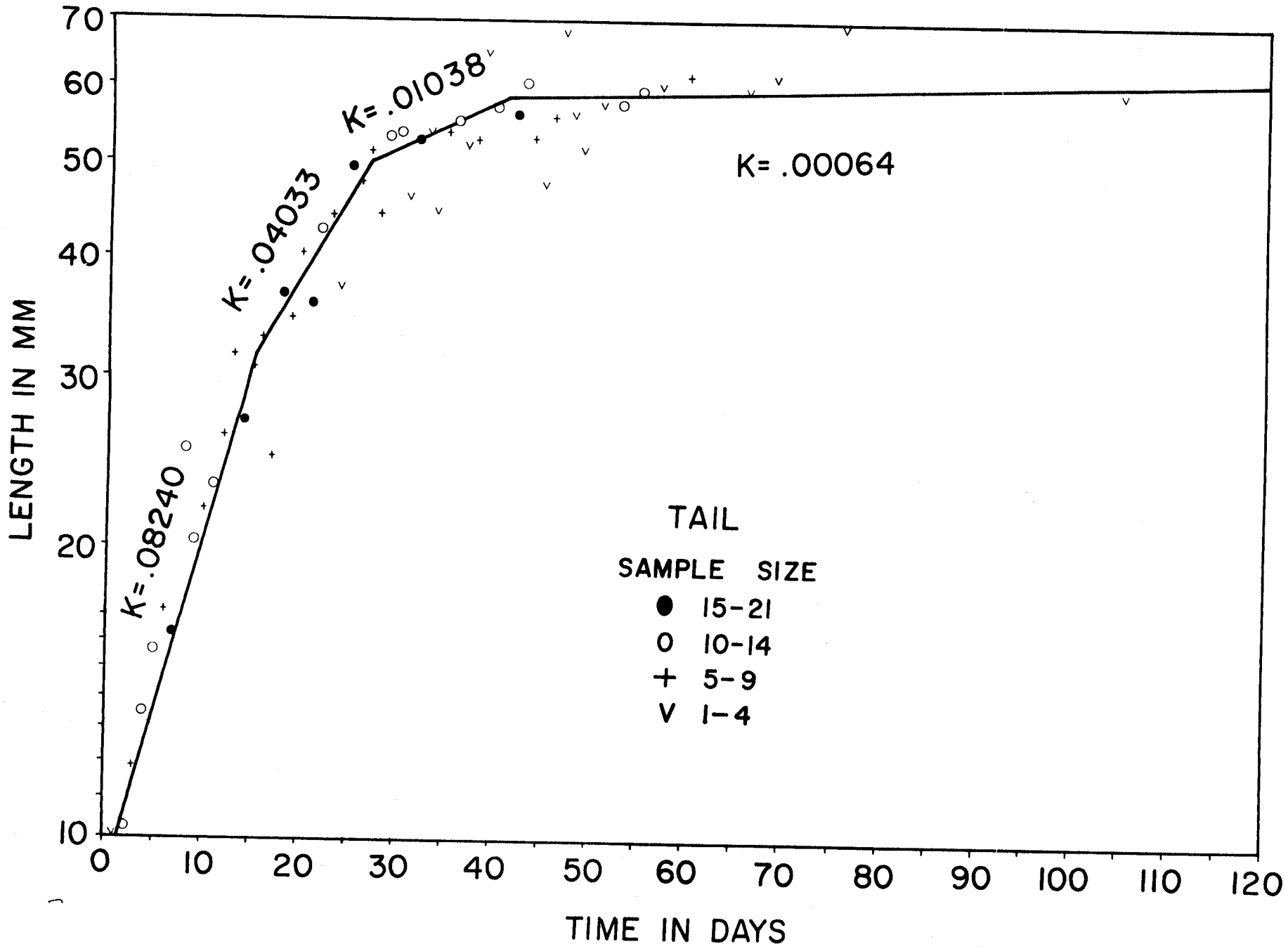

WEIGHT IN GRAMS
TIME IN DAYS
K=.07367
K=.02661
K=.00377
K=.00019
WEIGHT
SAMPLE SIZE
15-21
10-14
5-9
1-4

Fig. 3 - Semilogarithmic plots of total body and tail length of *P. longimembris*.


LENGTH IN MM
200
150
100
70
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
TIME IN DAYS
K=.04413
K=.03540
K=.00520
K=.00083
TOTAL BODY
SAMPLE SIZE
● 15-21
O 10-14
+ 5-9
V 1-4


LENGTH IN MM
TIME IN DAYS
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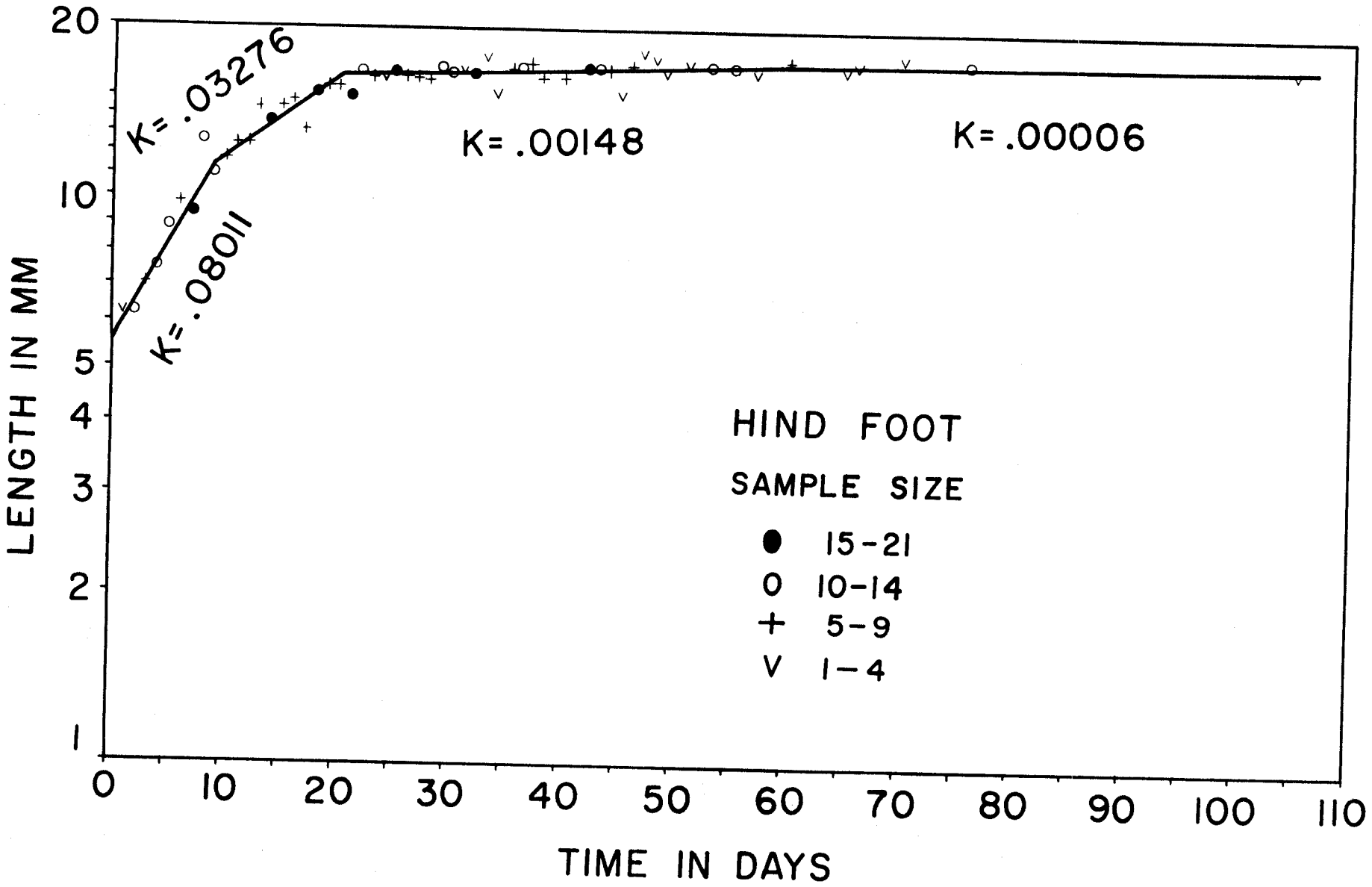
K= .04033
K= .01038
K= .00064
TAIL
SAMPLE SIZE
15-21
10-14
5-9
1-4
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70
80
90
100
110
120

Fig. 4 - Semilogarithmic plots of hind foot and ear length of *P. longimembris*.


20
10
5
4
3
2
1
LENGTH IN MM
K= .03276
K= .08011
K= .00148
K= .00006
HIND FOOT
SAMPLE SIZE
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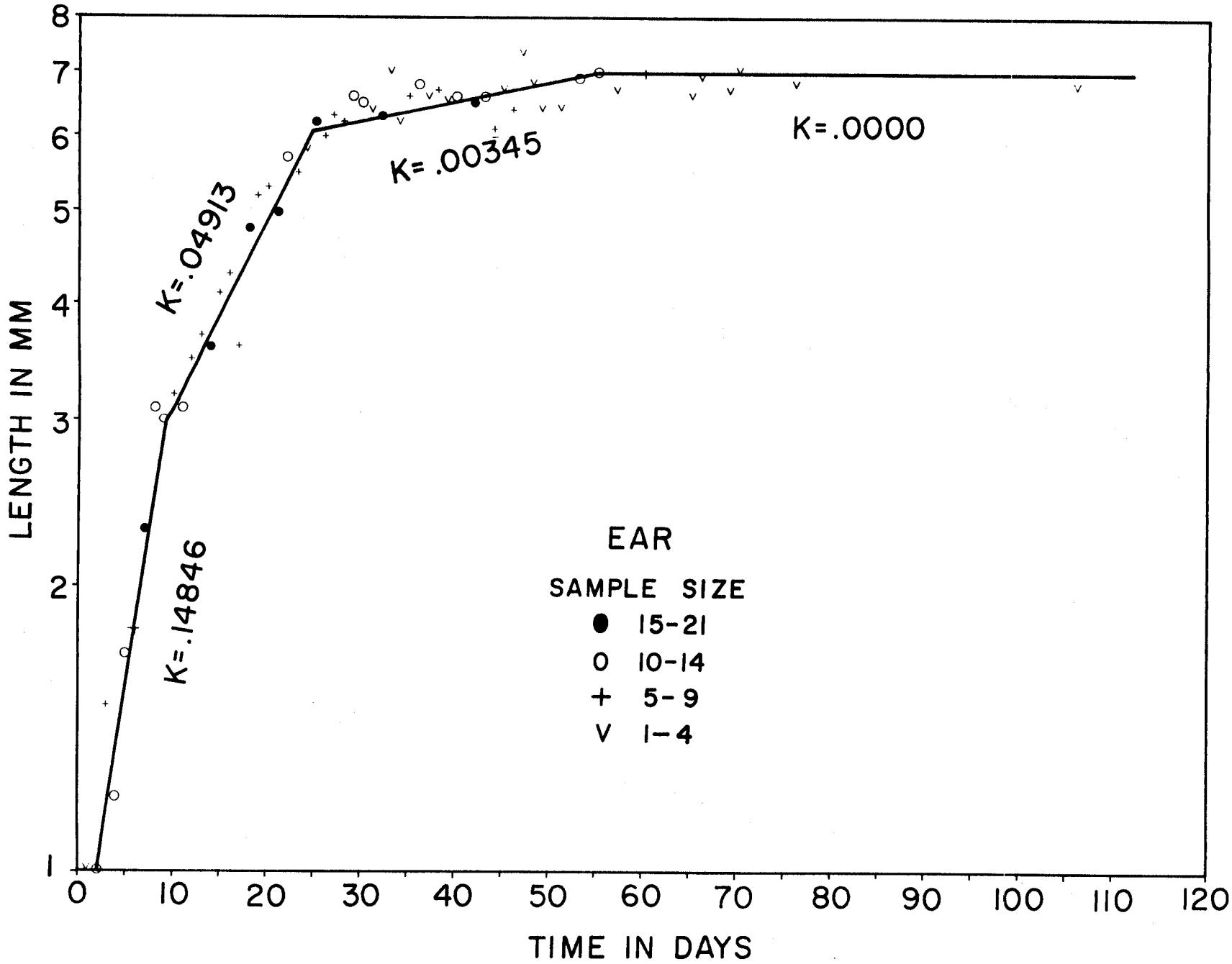
10-14
5-9
1-4
0
10
20
30
40
50
60
70
80
90
100
110
TIME IN DAYS


LENGTH IN MM
TIME IN DAYS
K=.14846
K=.04913
K=.00345
K=.0000
EAR
SAMPLE SIZE
15-21
10-14
5-9
1-4

RADIOBIOLOGY

The Radiobiology of the Pocket Mouse

J. J. Gambino and R. G. Lindberg

THE RADIOBIOLOGY OF THE POCKET MOUSE

J. J. Gambino and R. G. Lindberg

INTRODUCTION

For the past four years the pocket mouse genus, Perognathus, has been under intensive investigation in this laboratory. A primary objective of this work was to determine the feasibility of using pocket mice in biological experiments in space, and as a result, a substantial part of the research was concerned with radiation effects. Historically, our radiobiological investigations of pocket mice consisted of: (1) radiation response studies, and (2) mechanisms of radiation resistance studies.

The radiation response studies characterized the biological response of Perognathus spp. to conventional x-ray and Co^{60} ionizing radiation sources. It was ascertained that pocket mice withstand whole body doses of irradiation well above that tolerated by any other mammalian species (5). For example, 1000 R was formerly considered the $LD_{100(30)}$ for even the most resistant mammalian species; however, pocket mouse studies raised that limit to about 1500 R.

Our interest next focused on ways and means of elucidating the mechanism, or mechanisms by which pocket mice attain such a high degree of radiation resistance. There were two obvious approaches to the problem. One was to assume the presence of a very basic cellular peculiarity,

such as a unique enzyme system or chromosome make-up which enabled pocket mice to withstand high dose irradiation. The other possible approach was to examine the phenomenon at the total organism level, making the assumption that radiation resistance in this species results coincidentally from one or more physiological adaptations related to the animal's rather unique "mode-of-living".

For pragmatic reasons, we chose the latter approach and began studying the effect of various physiological factors on radiation response in this species. That work is summarized in a paper entitled "A Search for Mechanisms of Radiation Resistance in Pocket Mice" (6). As the title implies, the search continues. Studies of metabolic rate effects, hypoxia, hypothermia, tissue oxygen tension, and other factors known to influence radiation response in mammals, failed to uncover any obvious mechanism of radiation resistance in pocket mice.

However, despite the somewhat negative aspects of these experiments, they helped to crystallize, untimately, what appears to be the most promising experimental approach. It is based on the premise that total body response to radiation reflects radiosensitivity or radioresistance in critical regenerative cells. Complex multicellular animals either succumb or recover from radiation injury, depending upon whether a critical number of regenerative cells in vital tissues survives (17). Highly active regenerating tissues, such as bone marrow and intestinal epithelium of mammals, are particularly vulnerable to radiation damage, thus making mammals as a group extremely sensitive to irradiation. Abundant evidence is accumulating to suggest that variable radiosensitivity among the mammals may be based upon differences in cell population kinetics in hemopoietic and intestinal mucosal cell regenerative systems. Apparently, in pocket

mice the ratio of resistant cells to sensitive cells in these vital tissues is high.

Our attention then turned to cell renewal systems in the pocket mouse. We began work on the normal histology and post-irradiation pathology of pocket mouse intestine. We also initiated studies of mitotic activity in intestinal crypts, intestinal mucosa cell turn-over time, villus transit time, and bone marrow. Results to date indicate that the gastrointestinal syndrome in pocket mice is delayed or ameliorated by virtue of a very slow transit time of intestinal mucosal cells. Just how much of the observed radiation resistance of pocket mice is based on intestinal "protection", and how much on hemopoietic "protection" is still unknown. But certainly, an extremely radioresistant hemopoietic system must be postulated to explain the very high survival rates after whole body doses up to 1400 R.

Most of the work mentioned above has been either published in the open literature (4,5,6) or reported in the form of progress reports to NASA (7,9-14). The following pages summarize our knowledge of pocket mouse radiobiology and suggest future basic research utilizing some of the unique radiobiological characteristics of this species.

I. ACUTE EFFECTS OF IRRADIATION IN THE POCKET MOUSE

Pocket mice withstand single, whole body doses of X- and gamma irradiation that normally kills most other mammals within the 30-day acute post-irradiation period. Perognathus longimembris has an $LD_{50(30)}$ of 1520 R and P. formosus 1300 R. In the dose range between 3000 R and 10,000 R, the "gut-death" plateau is about 8 days. At doses above 10,000 R "CNS-death" occurs as in other mammals, with a mean survival time of about one day following 24.1 KR.

A number of physiological parameters were investigated as possible mechanisms of pocket mouse radiation resistance. As mentioned above, these studies did not clearly delineate any one mechanism of radiation resistance. We were able to eliminate several possibilities, however. For example, irradiation of pocket mice while hypometabolic, after splenectomy, or while in 100% oxygen at 3 atm. did not alter their response. On the other hand, irradiating the animals while in a 2.6% oxygen gaseous environment protected against the lethal effects of 2100 R. Preventing irradiated pocket mice from becoming hypometabolic during the 30-day acute period following exposure did not enhance lethality. Nor was survival altered by administering the irradiation to hypometabolic mice. It appears, then, that pocket mouse radiation resistance does not involve a tissue oxygen-related mechanism, nor does it appear to be related to the ability of pocket mice to undergo hypothermia.

As stated in the introduction, whole body radiosensitivity may be closely related to cell population dynamics in critical radiosensitive tissues. Cells vary in their response to irradiation. One factor affecting the response of a cell is time of administration of irradiation relative to the cell cycle. It follows that differential sensitivity of tissues may simply reflect the ratio of cells in a radiosensitive phase to those in a resistant phase. Since in mammalian radiobiology, survival or death after whole body irradiation usually depends upon the degree of injury or repair in specific tissues and organs, the entire question of radiation resistance may be answered by a meticulous study of mitotic activity in the tissues of interest.

A number of strains of laboratory mice and rats exhibit distinctly different survival times when irradiated at various times of the diurnal activity cycle (18). If the hypothesis relating species' difference in whole body radiation sensitivity to cell renewal system dynamics has merit, it should also explain intraspecific diurnal differences in radiosensitivity. It is possible that these diurnal differences may be correlated with mitotic activity and cell cycles in bone marrow and intestine, since rhythmic mitotic activity has been reported in a number of mammalian tissues. Unfortunately, sufficient data is not available to judge this hypothesis in conventional mice and rats. Indeed, available evidence indicates there is no rhythmic mitotic activity in the intestines of these species (1,15).

On the other hand, we have evidence that a peak of mitotic activity occurs in the intestinal crypts of pocket mice during one part of their diurnal cycle. In this study, animals were sacrificed at 4-hour intervals over a 28-hour period. Mitotic activity was estimated by counting the

number of mitotic figures in three complete cross sections of jejunum for five mice sacrificed at each time interval (Figure 1). The peak of mitotic activity occurred at 0400 hours Pacific Time, which is nearing the end of the metabolic high period for Perognathus longimembris.

Previously we had shown that P. longimembris responds differently to irradiation administered at two different times of the day (9). A significantly higher mean survival time was noted in animals irradiated at 2330 hours, in contrast to those irradiated at 0900 hours. The experiment was designed to deliver the irradiation at the middle of the metabolic low and high periods based on prior observations of locomotor activity and studies of oxygen uptake (Figure 2).

In summary, P. longimembris exhibits well-defined daily periodicity in body temperature, metabolic rate and locomotor activity. These interrelated parameters have been measured and reported from our laboratory (3,13,14). We have noted a prolonged intestinal epithelial cell transit time, and we have also seen a peak of mitotic activity in intestinal crypt cells during one part of their diurnal cycle. In addition, we have demonstrated different survival rates in pocket mice irradiated at two different times of the day. Although preliminary, these results suggest a close relationship between photoperiod, diurnal rhythms and radiation response in this species. We feel that interspecific and intraspecific differential radiosensitivities are based on the same physiological mechanisms and can be better understood by further studies of cell renewal systems in radioresistant mammals.

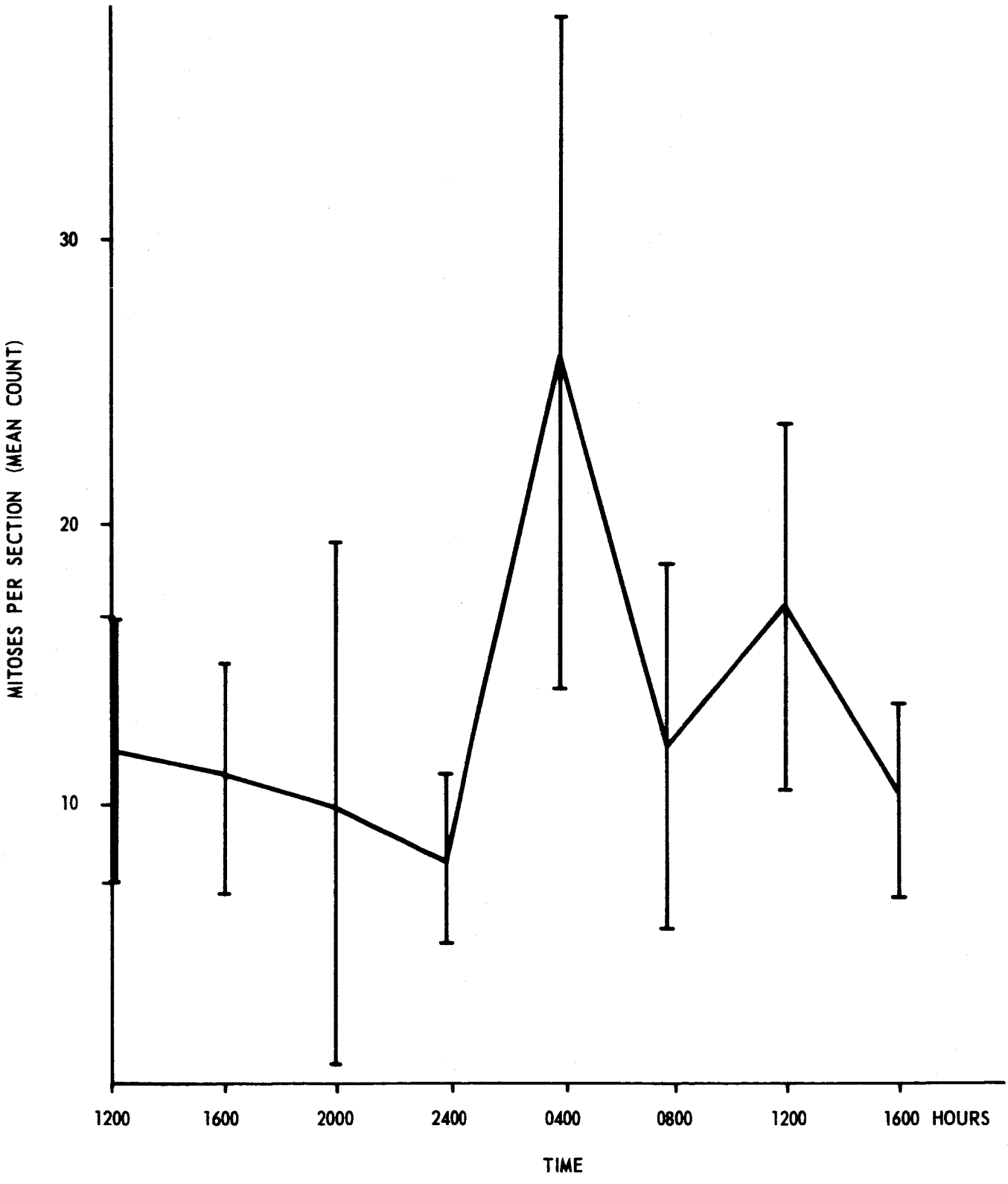


FIGURE 1 MITOTIC ACTIVITY OF INTESTINAL CRYPT CELLS OF PEROGNATHUS LONGIMEMBRIS. ACTIVITY IS ESTIMATED BY NUMBER OF MITOTIC FIGURES IN THREE COMPLETE CROSS SECTIONS OF 5 MICE SACRIFICED AT EACH TIME INTERVAL.

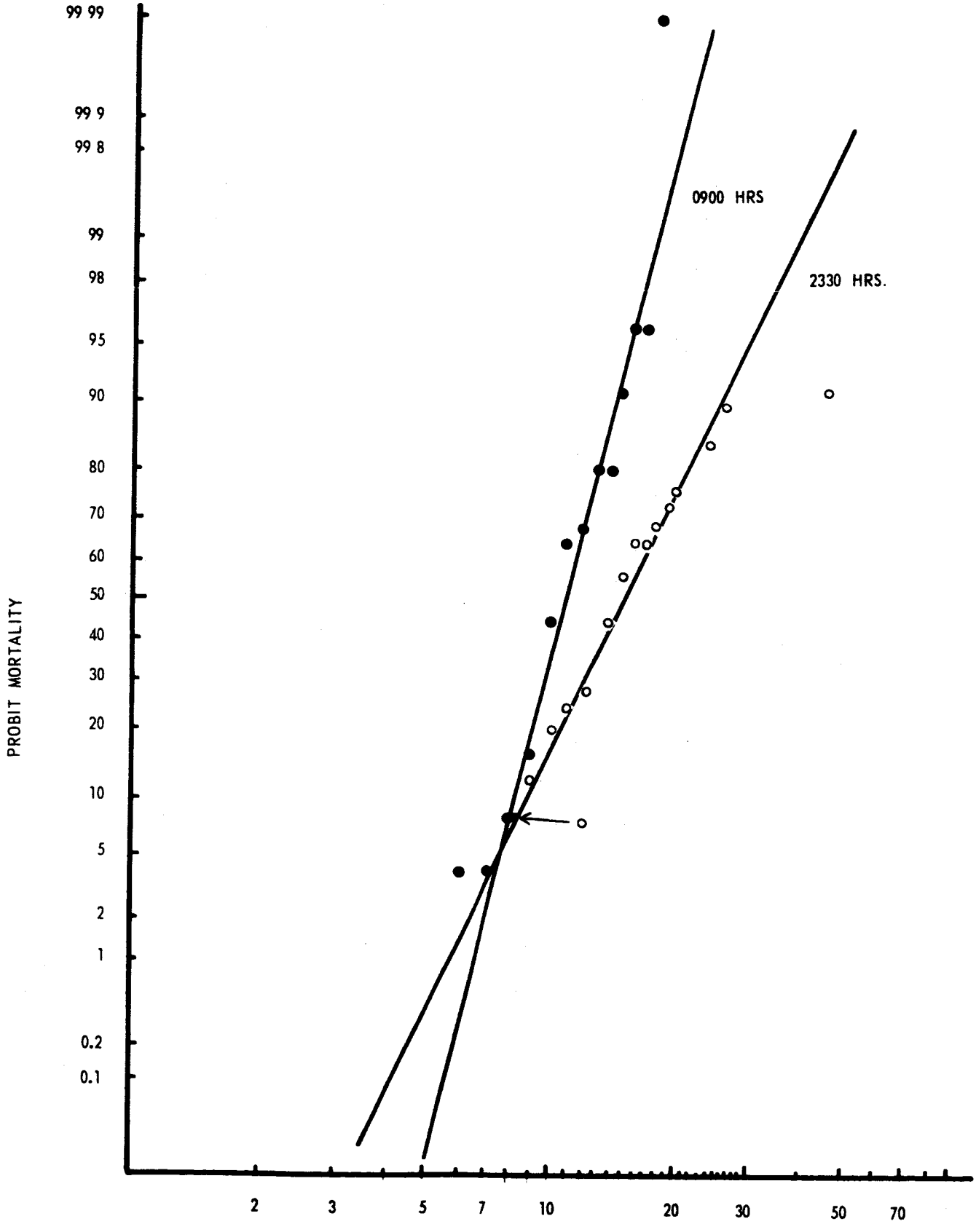


FIGURE 2 PROBIT TRANSFORMATION OF SURVIVAL DATA OF POCKET MICE ADMINISTERED 1500 RADS CO^{60} RADIATION AT TWO TIMES OF DAY

II. LATE EFFECTS OF IRRADIATION IN POCKET MICE

Late effects of irradiation in pocket mice are superficially similar to other mammals. Survivors of high dose radiation exhibit graying, premature "aging", and life shortening. Since pocket mice are long-lived animals, conclusive statistical data are not yet available; however, there is presently sufficient evidence to suggest that the late effects response of pocket mice is somewhat peculiar.

For example, except for graying, the onset of late changes appears to be disproportionately delayed in this species as compared with conventional mice and rats given the same dose of irradiation. This delay is apparent at three years post-irradiation in the low incidence of cataracts, tumors, glomerulosclerosis and other nonspecific diseases associated with long-term effects of irradiation.

Mortality curves of irradiated and control P. longimembris are similar to about two and one-half years post-irradiation (Figure 3). Beyond that time, the highest dose groups show an increased mortality rate. Visual inspection of these mortality curves suggests that 1000 R whole body irradiation decreases the life expectancy of a pocket mouse by about one-third.

Graying in pocket mice, as in other species, is dose dependent. Animals receiving doses above 1000 R become completely gray at the first molt following exposure. Animals that received 600 and 800 R show no coat color changes until about one year after exposure. By three years post-

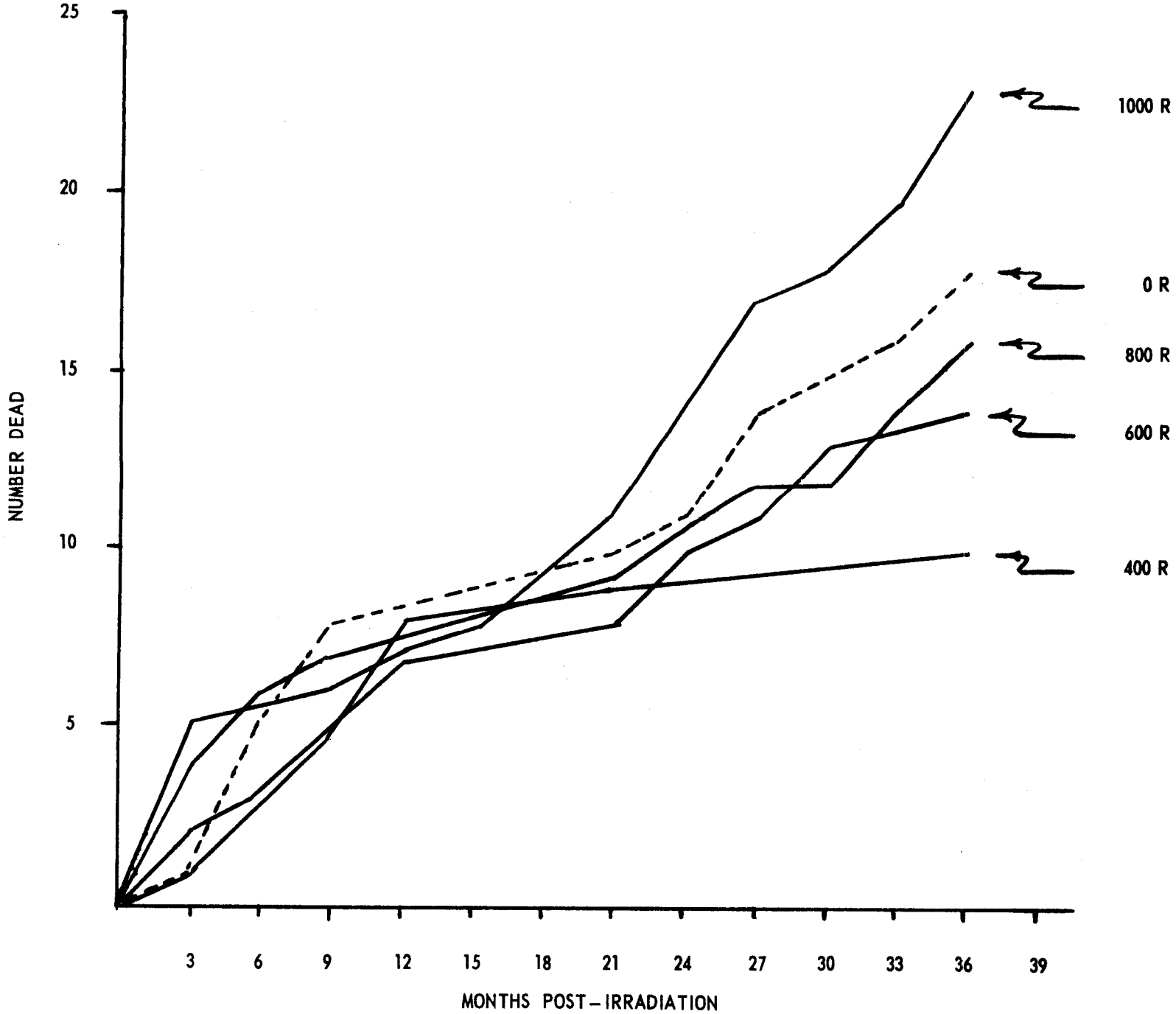


FIGURE 3 MORTALITY OF PEROGNATHUS LONGIMEMBRIS AFTER VARIOUS DOSES OF X-IRRADIATION. INITIAL NUMBER EACH GROUP - 25.

irradiation, the 800 R animals are totally white, while coat color in the 600 R animals is best described as grayish-brown. Animals receiving 400 R exhibit no coat color changes even at three years post-irradiation.

There are presently about 160 P. longimembris in our facility being closely watched for late effects. This number represents the survivors of nearly 400 mice that were irradiated in this laboratory over the past three years and their controls. Of the 160 remaining mice, 66 are unirradiated controls. The colony is inspected routinely for clinical signs of disease. When an animal is found dead, it is autopsied, and the entire carcass is fixed in Bouin's solution. Subsequently, pathology slides are prepared of representative tissues. Histopathological examination has revealed no pathology that can be considered a late consequence of irradiation. Animals that have been examined include some which received radiation doses ranging from 400 to 1600 R two to three years prior to their death.*

Extrapolation of mortality curves indicates that the life span of normal pocket mice in captivity may be as long as five to six years. This means that a final analysis of late effects of irradiation in pocket mice will not be available until 1967 or 1968.

*We wish to acknowledge the capable participation of Dr. G. E. Cosgrove and W. D. Gude of the Biology Division, Oak Ridge National Laboratory in this phase of the work.

III. BASIC RADIOBIOLOGICAL RESEARCH WITH POCKET MICE

In mammalian radiobiology it is reasonable to use experimental organisms that best illustrate mechanisms of injury or repair in specific radiosusceptible tissues. The experimental animal of choice need not be one more closely related to man, but one that has particular physiological processes that are amenable to the kind of study necessary for a better understanding of man (20). Recent use of mammalian species other than conventional laboratory forms for comparative radiobiology has shown considerable promise toward elucidating basic mechanisms of radiation injury and recovery in man (2,8).

Pocket mice are particularly interesting subjects for basic radiobiological studies. They appear to be ideally suited for experiments designed to elucidate interrelationships of physiological rhythms, mitotic rhythms, and radiation response.

One example of the kind of experiment that could be easily performed with this species is to determine the effect of shifting the photoperiod on radiation response. If rhythms in cell renewal systems are correlated with metabolic or other physiological rhythms, which in turn are cued by photoperiod, some very interesting changes in radiation response might be anticipated by shifting photoperiod 180 degrees prior to irradiation.

The obvious insensitivity of pocket mouse hemopoietic stem cells to irradiation suggests numerous studies which would provide a better understanding of the role of bone marrow damage in the acute radiation syndrome.

It would be very interesting, for example, to use the technique of McCulloch and Till (16) to determine the D_{37} for pocket mouse marrow cells and to correlate this with persistent chromosome aberrations in circulating leucocytes (19), comparing the results with those reported for conventional mice.

The insensitivity shown by Perognathus to ionizing electromagnetic radiation raises the question of its response to particulate irradiation. It has been shown that with high LET radiations gastrointestinal deaths predominate, while with X- and gamma radiation marrow deaths abound. Since the hemopoietic system of pocket mice appears to be extremely resistant to X- and gamma irradiation, exposing Perognathus to particulate irradiation may provide a means of discerning the relative importance of these two modes of death in this species. Particulate beam irradiation of pocket mice may also provide clues to the basis of radiation resistance in this species, by virtue of the fact that response to high LET irradiation is independent of tissue oxygen tension.

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